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THE MATING SYSTEM OF LODGEPOLE PINE IN ALBERTA

by

DANIEL JAMES PERRY

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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Abstract

The mating systems of three widely separated lodgepole pine (*Pinus contorta* var. *latifolia* Dougl.) populations in the Rocky Mountain foothills of Alberta were estimated. The population multilocus outcrossing rate estimates ranged from 0.926 to 0.983. No significant differences in outcrossing rates were found among stands, among seed years within stands, or among crown positions within trees. However, a tentative temporal trend is suggested in which selection against inbred seed is occurring over time in the seed pool retained on the tree. Significant heterogenity was found among single-tree outcrossing estimates in one stand.

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I. INTRODUCTION

The mating system is an important determinant of plant population genetic structure (Clegg, 1980). It is the genetic link between generations and determines the initial zygotic frequency distribution of each generation. Individuals of a highly outcrossed species generally have higher levels of heterozygosity than those of self-fertilizing species (Brown, 1979). Self-fertilization can also induce linkage disequilibrium even among loci on different chromesomes (Allard, 1975; Mitton *et al.*, 1981).

Knowledge of the mating system is also of practical significance for forest tree breeders and those involved in reforestation. Selfed progeny of coniferous forest species display significant inbreeding depression in the form of decreased survival and growth (Franklin, 1970; Sorensen and Miles, 1982). In addition, the presence of inbreeding violates the assumptions made in analysis of wind-pollinated progeny tests, leading to overestimation of additive genetic variance and genetic gain (Namkoong, 1966; Squillace, 1974).

Several methods have been employed in the study of mating systems of forest trees. Earlier estimates of outcrossing rates were largely based on mutant phenotype frequencies (Squillace and Kraus, 1963; Fowler, 1965; Franklin, 1971a; Sorensen, 1973) or on the percentage of filled seed (Franklin, 1971b). With the advent of gel electrophoresis, allozyme polymorphisms have become commonly used gene markers for examining the mating systems of forest

trees, because they provide many marker loci and more alleles per locus, increasing the statistical precision of estimates (Moran and Brown, 1980; Fripp, 1982; Yeh *et al.*, 1983; King *et al.*, 1984; Farris and Mitton, 1984; Epperson and Allard, 1984; Furnier and Adams, 1985). Single locus (Brown *et al.*, 1975; Shaw and Allard, 1982; Cheliak *et al.*, 1983) and multilocus models (Green *et al.*, 1980; Ritland and Jain, 1981; Shaw *et al.*, 1981) have been developed to estimate mating system parameters from allozyme data. These methods give an estimate of t, the proportion of viable progeny due to outcrossing (outcrossing rate).

Gymnosperms are particularily convenient for electrophoretic studies. The haploid megagametophyte tissue is genetically identical to the maternal contribution to the embryo. Therefore, by comparing the electrophoretic phenotype of the megagametophyte to that of the corresponding embryo, one may infer the paternal contribution at a given locus. This information may then be applied to an analysis of the mating system.

Coniferous species with serotinous cones provide a good opportunity to study temporal variation in the mating system, because they can retain seed within their cones for a number of years. Therefore, it is possible to collect seed from a number of different seed crops simultaneously.

In this study, both spatial and temporal aspects of the mating systems of three widely separated populations of lodgepole pine (*Pinus contorta* var. *latifolia* Dougl.) in

Alberta were examined through allozyme analysis. The objective was to determine whether the mating system of lodgepole pine varies among stands, among trees within a stand, among crown positions within a tree, and over time.

II. METHODS

Seed Collection

Cones were collected from three widely separated, natural, even-aged lodgepole pine stands in the Rocky Mountain foothills of Alberta during the summer of 1983 (Table 1). A square grid of 49 10m x 10m plots was laid out in each stand. Sample trees were selected in the 25 plots falling on the median and diagonal axes. The lodgepole pine tree closest to the center of the plot and having an adequate cone crop was sampled.

Five subsamples of cones were collected from most sample trees. Bulk upper crown (UC) and bulk lower crown (LC) cone subsamples were taken randomly with respect to cone age from the upper third and lower third of the reproductive crown. First (W1), third (W3) and fifth cone whorl (W5) subsamples were collected from the upper portion of the crown. In doing so, branches which obviously had multiple whorls of cones in past years were avoided. It was not possible to collect W5 samples from a few trees, because they did not have sufficient branches with cone whorls one through five intact. Seed was extracted and stored separately by tree and subsample at 4°C until required for analysis.

Table 1. Description of lodgepole pine collection sites in the Rocky Mountain foothills of Alberta.

Population	Latitude	Longitude	Elevation (m)	Average Age (years)	Basal Area (m² / ha)
Robb	53°10′	117°15′	1300	84.2	11.85
Nordegg	52°30′	116°04′	1100	80.1	10.89
Cochrane	51°40′	115°12′	1300	89.3	12.55

Electrophoretic Methods

Seed was germinated in petri dishes until the radicle had emerged at least 2mm. The haploid megagametophyte and diploid embryo from approximately 10 seeds per subsample in each family were individually homogenized in 50μl of extraction buffer (Yeh and Layton, 1979) and assayed for six allozyme loci using starch gel electrophoresis (12.5% Connaught Hydrolysed Starch) (Table 2). The loci were chosen because they were consistently well resolved, polymorphic loci in which the embryo genotype could be interpreted unambiguously when compared to the corresponding megagametophyte. Inheritance and linkage relationships have previously been determined for these loci in lodgepole pine (Conkle, 1981; Yeh, unpublished data, Research Branch, British Columbia Forest Service, Victoria).

Statistical Methods

Genotypes of individual mother trees were inferred at the six loci based upon allozyme segregation in 35 to 50 megagametophytes per tree (mean = 49). With 35 megagametophytes per tree the probability of misclassifying a maternal genotype at any one locus is exceedingly small (5.82×10^{-11}).

Single-locus (t_s) and multilocus (t_m) estimates of outcrossing rates and estimates of outcross pollen pool allele frequencies were obtained using the maximum likelihood procedures and computer programs of Neale and

Table 2. Enzyme systems assayed in seed tissue of lodgepole pine.

Locus	E.C. Number	Buffer System[1]	Stain Reference
Aconitase (ACO)	4.2.1.3.	A	Yeh and O'Malley (1980)
Alcohol dehydrogenase (ADH)	1.1.1.1.	B	Shaw and Prasad (1970)
Isocitrate dehydrogenase (IDH)	1.1.1.41.	A	Yeh and O'Malley (1980)
Phosphoglucomutase (PGM)	2.7.5.1.	A	Yeh and O'Malley (1980)
Phosphoglucose isomerase (PGI-2)	5.3.1.9.	C	Yeh and O'Malley (1980)
6-Phosphogluconic dehydrogenase (6PG-1)	1.1.1.44.	C	Yeh and O'Malley (1980)

[1]Buffer systems:

A Tris-citrate pH 7.0 gel buffer; Tris-citrate pH 7.0 electrode buffer (Siciliano and Shaw, 1976). Run at 200 volts.

B Tris-citrate pH 8.5 gel buffer; lithium borate pH 8.1 electrode buffer (Ridgeway *et al.*, 1970). Run at 300 volts.

C L-histidine-tris pH 7.0 gel buffer; Tris-citrate pH 7.0 electrode buffer (Florence, 1981). Run at 200 volts.

Adams (1985). Maximum likelihood estimators for t_s and t_m were described in Shaw and Allard (1982) and Green *et al.* (1980), respectively. To estimate t_s for loci with four alleles (ACO and ADH), it was necessary to combine the two least common alleles into a third synthetic class. Since a minimum of two maternal genotypic classes and a variable pollen pool were required to estimate t_s, it was not possible to obtain estimates for all loci in all subsamples. A likelihood ratio test (Rao, 1973) was used to test the hypotheses H_0: $t_s = 1$ and H_0: $t_m = 1$ for the population estimates. Single-tree estimates of t_m were obtained for a random sample of six trees per population. Differences among outcrossing rates of populations, crown positions, cone whorls and individual trees were tested using an H (homogeneity) test (Rao, 1973).

Both t_s and t_m estimators rely upon the mixed mating model (Fyfe and Bailey, 1951; Shaw and Allard, 1982), which assumes that each of the observed progeny is a result of either a random outcross (with probability t) or self-fertilization (with probability $1 - t = s$), that no selection occurs between germination and analysis of progenies, that the genotype of the maternal parent does not influence the probability of an outcross progeny, and that allele frequencies in the outcross pollen pool are homogeneous among maternal parents (Shaw et al., 1981). Independence among loci in the outcross pollen pool must also be assumed for multilocus estimation.

Wright's fixation index (F) (Wright, 1951), a measure of the deviation of observed heterozygote frequency from that expected under Hardy-Weinberg equilibrium, was calculated for filial populations as:

$$F = 1 - H/h,$$

where H is the observed proportion of heterozygous individuals and h is the proportion of heterozygotes expected under panmixia. Expected heterozygosity was calculated as:

$$h = 1 - \Sigma p_i^2,$$

where p_i is the estimated frequency of the *i*th allele at the locus.

To test for spatial heterogeneity of outcross pollen allele frequencies, 2 x m contingency tables were constructed, where m was the number of maternal trees of one homozygous genotype in a population. The number of detectable outcrosses (heterozygotes) was compared with homozygous progeny through the use of heterogeneity chi-square analysis (Brown *et al.*, 1975). Similarly, spatial (tree and position within crown) and temporal (cone whorls) effects were investigated for heterogeneity by contingency table analysis (Sokal and Rohlf, 1981).

All tests of significance in this study were conducted at the $p < 0.05$ level.

III. RESULTS

Estimates of t_s ranged from 0.768 to 1.401 (Table 3), with significant heterogeneity among loci in five of the 15 subsamples and two of the three populations when subsamples were pooled. Population estimates of t_m ranged from 0.858 to 1.022 (Table 4), with no significant differences among estimates for different cone whorls or crown positions within a population. Despite the lack of significant differences, UC subsamples consistently had slightly lower t_m estimates than LC subsamples. No trends were apparent among whorls (W1, W3, W5). Due to the lack of heterogeneity among subsamples, estimates based on data pooled across subsamples within a population were used as the best population estimates of t_m. These estimates ranged from 0.926 to 0.983 and were not significantly heterogeneous. Estimates of t_m for the Cochrane (0.926) and Robb (0.934) populations were significantly different from 1.0, while the estimate for the Nordegg population (0.983) was not.

Although there was a wide range in individual tree estimates of t_m within a population (e.g., 0.641 to 1.090 in the Robb population), significant heterogeneity among estimates occurred only in the Nordegg population (Table 5).

No significant deviations from Hardy-Weinberg genotype frequencies occurred at ACO, IDH, 6PG-1, PGI-2 or PGM in any of the filial subsamples. Significant deviations were observed in two subsamples (Robb UC and Robb W3) at the ADH locus. When subsamples were pooled, significant deviations

Table 3. Population single-locus outcrossing estimates (ts) and sample sizes for upper (UC) and lower (LC) crown, first (W1), third (W3), and fifth (W5) cone whorls, and pooled subsamples for three lodgepole pine populations in Alberta.

Population	Subsample	n	Locus						
			ACO	ADH	IDH	6PG-1	PGI-2	PGM	Mean[3]
Robb	UC[1]	250	0.864[1]	0.795[1]	-	1.052	1.033	-	0.936
	LC[1]	250	0.960	0.768[1]	0.917	-	1.033	1.025	0.941
	W1	240	0.940	1.037	1.009	0.918	1.043	-	0.989
	W3[1]	245	0.861[1]	0.892[1]	1.018	1.036	0.834	1.017	0.943
	W5[1]	250	0.899	1.401[1]	1.017	1.043	1.042	1.033	1.073
	Pooled[2]	1235	0.904[1]	0.911[1]	0.992	1.010	0.997	1.023	0.973
Nordegg	UC	250	0.978	-	1.017	-	-	1.033	1.009
	LC	250	1.055	-	1.025	-	-	-	1.040
	W1	240	0.856[1]	0.963	1.017	-	-	1.009	0.961
	W3	250	0.933	0.974	-	-	-	-	0.953
	W5	240	0.927	1.003	1.026	-	-	1.017	0.993
	Pooled[2]	1230	0.954	0.980	1.017	-	-	1.012	0.991
Cochrane	UC	240	0.871[1]	0.850	-	0.886	-	0.971	0.895
	LC[1]	247	0.880[1]	0.884[1]	-	1.091	-	-	0.952
	W1	241	0.944	0.934	-	0.948	-	0.948	0.944
	W3	240	0.993	0.972	-	1.005	-	0.943	0.975
	W5	240	0.932	0.971	-	0.973	-	-	0.959
	Pooled	1208	0.922[1]	0.928[1]	-	0.987	-	0.989	0.957

[1]Significant ($P < 0.05$) departure from H_0: ts = 1.0 (test not made for means).

[2]Significant ($P < 0.05$) heterogeneity among estimates within subsample.

[3]Unweighted mean over loci.

Table 4. Population multilocus outcrossing estimates, t_m (standard errors in parentheses), for upper (UC) and lower (LC) crown, first (W1), third (W3), and fifth (W5) cone whorls, and pooled subsamples for three lodgepole pine populations in Alberta.

	Population		
Subsample	Robb	Nordegg	Cochrane
UC	0.858' (0.059)	1.014 (0.074)	0.899' (0.052)
LC	0.874' (0.061)	1.022 (0.073)	0.907 (0.054)
W1	0.948 (0.061)	0.935 (0.049)	0.907 (0.057)
W3	0.961 (0.061)	0.984 (0.047)	0.977 (0.051)
W5	1.007 (0.054)	0.984 (0.046)	0.934 (0.053)
Pooled	0.934' (0.027)	0.983 (0.021)	0.926' (0.024)

'significant ($p < 0.05$) departure from H_o: $t_m = 1.0$.

Table 5. Single-tree multilocus outcrossing estimates, t_m (standard errors in parentheses), for three lodgepole pine populations in Alberta.

Population	Tree Number	t_m
Robb	7	0.641 (0.277)
	11	0.739 (0.150)
	17	1.090 (0.222)
	23	0.993 (0.140)
	24	0.875 (0.116)
	39	1.025 (0.078)
Nordegg'	1	1.064 (0.139)
	7	0.745 (0.158)
	11	0.840 (0.120)
	13	1.188 (0.126)
	17	0.679 (0.114)
	24	1.146 (0.398)
Cochrane	4	0.796 (0.109)
	18	1.117 (0.120)
	27	0.962 (0.099)
	28	0.788 (0.134)
	32	1.110 (0.272)
	43	0.901 (0.053)

'estimates significantly heterogeneous ($p < 0.05$) among trees within population.

occurred only at ADH in the Robb population and ACO in the Cochrane population. All significant deviations from Hardy-Weinberg genotype frequencies were due to an excess of homozygotes, as indicated by the positive F values in these cases (Table 6). Another consistent trend that is suggested by the F estimates at both ACO and ADH is an apparent decrease in F from UC to LC subsamples within a population.

Pollen pool allele frequencies did not differ significantly among trees within a population, nor among cone whorls or crown positions. Although not statistically significant, tentative trends were observed. In contingency models involving trees and cone whorls as main sources of variation, an average of 26% (range 17.6% to 33.7%) of the variation resided among trees, while an average of 14.3% (range 9.4% to 21.5%) was among cone whorls. An average of 37.8% (range 25.2% to 70.3%) of variation was found among trees and an average of 2.6% (range 0.0% to 9.6%) among crown positions in models containing trees and crown positions as main sources of variance. Among-tree variation was always greater than either among-cone-whorl or among-crown-position variation. Such contingency analyses were carried out only for ACO and ADH, because the other loci had too few heterozygous embryos for valid testing.

Table 6. Estimates of Wright's Fixation Index (F) for filial subpopulations from three lodgepole pine populations in Alberta.

Population	Subsample	Locus					
		ACO	ADH	IDH	6PG-1	PGI-2	PGM
Robb	UC	0.067	0.123'	-0.016	-0.033	-0.017	-0.008
	LC	0.040	0.101	0.107	-0.025	-0.018	-0.012
	W1	0.031	0.006	-0.017	-0.026	-0.023	-0.011
	W3	0.084	0.073'	-0.032	-0.032	-0.019	-0.004
	W5	-0.031	-0.019	-0.025	-0.027	-0.025	-0.016
	Pooled	0.038	0.060'	-0.011	-0.028	-0.020	-0.010
Nordegg	UC	-0.018	0.071	-0.010	-0.016	-0.009	-0.014
	LC	-0.023	0.014	-0.016	-0.018	-0.013	-0.012
	W1	0.080	0.053	-0.014	-0.013	-0.011	-0.006
	W3	0.061	0.061	-0.012	-0.010	-0.012	-0.010
	W5	-0.005	0.052	-0.019	-0.013	-0.002	-0.012
	Pooled	0.019	0.055	-0.014	-0.014	-0.009	-0.011
Cochrane	UC	0.090	0.075	-	-0.055	-0.006	0.061
	LC	0.051	0.066	-0.004	-0.049	-0.004	-0.036
	W1	0.072	0.116	-0.005	0.105	-0.015	-0.030
	W3	-0.087	0.122	-	-0.039	-	0.105
	W5	0.031	-0.015	-0.002	-0.054	-0.011	-0.041
	Pooled	0.032'	0.072	-0.002	-0.004	-0.007	0.012

'significant deviation ($p < 0.05$) from H_o: $F = 0.0$.

IV. DISCUSSION

The results of this study indicate that lodgepole pine populations in the Rocky Mountain foothills of Alberta are highly outcrossing. These results are in close agreement with those of Epperson and Allard (1984), in which t_m for two populations of lodgepole pine in northeastern Washington did not differ significantly from 1.0. High outcrossing rates have been found in the majority of conifers examined (Cheliak *et al.*, 1985; Furnier and Adams, 1985; King *et al.*, 1984; Mitton *et al.*, 1981; Shaw and Allard, 1982).

Significant heterogeneity among single-locus outcrossing estimates have often been observed in predominantly outcrossing plants (Brown and Allard, 1970; Brown *et al.*, 1975). Since in this study all estimates for a subsample or population were made from the same set of embryos, the observed variability must be due either to random variation or violations of the assumptions of the mixed-mating model (Shaw and Allard, 1982). Multilocus estimates of outcrossing are likely to be more accurate than single-locus estimates because they utilize a larger amount of information and are less succeptable to violations of assumptions of the mixed-mating model. Single-locus estimates of outcrossing are expected to be biased downward by consanguineous matings other than selfing (Ellstrand and Foster, 1983; Shaw and Allard, 1982). Such a bias was not evident in this study, in which the majority of means of single-locus estimates were larger than the corresponding

multilocus estimates. This observation, coupled with a lack of pollen pool heterogeneity among maternal trees, suggests a lack of family structuring within the maternal populations studied. This is contrary to the common assumption that most conifers, especially species with serotinous cones, have some degree of family structuring within populations (Libby *et al.*, 1969). However, relationships among maternal trees were not directly measured in this study. If such relationships had been determined, it is likely that family groups, if in existence, could remain undetected due to the wide spacing between sample trees. Lee (1985) found evidence of family structure within these same stands.

The differences among single-tree t_m estimates in the Nordegg population may be due to variable levels of self fertility. A prefertilization mechanism is unlikely, since cytological studies in lodgepole pine have shown that self-pollination does not reduce pollen germination, growth of the pollen tube or fertilization capacity (Hagman, 1964). Polyzygotic polyembryony, a post-fertilization factor, has been reported in the genus *Pinus* (Dogra, 1967), providing an opportunity for selection among embryos of unequal vigor during early embryo development (Sorensen, 1982). Therefore, variation among trees in the numbers of embryonic recessive lethals and deleterious alleles, or even in the degree of polyzygotic polyembryony, may account for the observed tree-to-tree variation in t_m. Such heterogeneity may complicate the analysis of data from wind-pollinated progeny

tests. Progeny of trees with low outcrossing rates may contribute to an overestimation of additive genetic variance and potential genetic gains and, if inbreeding depression is pronounced, could conceivably result in a maternal tree, which is genetically superior in other respects, being excluded from a breeding program.

Higher levels of outcrossing have been observed in seed collected from the upper crown than that from the lower crown in Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) (Shaw and Allard, 1982), jack pine (*Pinus banksiana* Lamb.) (Fowler, 1965), loblolly pine (*Pinus taeda* L.) (Franklin, 1971b), and Scots pine (*Pinus silvestris* L.) (Sarvas, 1962). This is usually attributed to a higher concentration of male strobili in the lower crown. Although no significant differences were found between upper and lower crown in this study, the results indicate a tentative trend contradictory to those previously reported, with lower outcrossing rates in the upper crown. However, since lodgepole pine's serotinous cones remain on the tree for many years, this tentative trend may be more accurately described as temporal rather than spatial. The observed degree of cone weathering indicated that the average age of cones from the lower crown was consistently much older than that of the upper crown. In fact, cones from the most recent cone year were rare in the lower reproductive crown. Most cones collected from the lower crown position likely occupied upper crown positions when originally formed.

Temporal heterogeneity has been found in mating systems of other forest tree species. Cheliak *et al.* (1985) found evidence of a temporal trend in jack pine, a closely related conifer, in which selfing apparently decreased with cone age. A similar trend was found by Moran and Brown (1980) for a population of alpine ash (*Eucalyptus delegatensis* R.T. Bak.), where significant differences in outcrossing rates of three seed crops were found, with the oldest crop having the highest outcrossing rate and the most recent having the lowest. Since both species retain their seed for many years, these temporal shifts have been attributed to selection against selfs in the embryo populations. The apparent decrease in F from upper to lower crown observed in the lodgepole pine populations studied here indicates such a mechanism may be operating, but may be obscured partially by the presence of some new seed in the bulk lower crown subsamples and some relatively old seed in the bulk upper crown subsamples. If selection against inbred seed is occurring over time in the seed pool retained on the tree, it is not strong enough to become evident during the time interval between the first and fifth cone-whorl subsamples. Since there was only a small initial proportion of selfed embryos available on which such a mechanism could operate, the time period necessary for the trend to be observable would likely be increased, and the chance of such a trend ever having statistical significance would be remote.

The high outcrossing rates observed in the lodgepole pine populations in this study may maintain genetic variation that may be required by a species to cope with a spatially and temporally heterogeneous environment (Campbell, 1979; Hedrick *et al.*, 1976). Of practical significance to foresters, the high observed outcrossing rates indicated that wind-pollinated lodgepole pine seed from extensive, even-aged stands with densities similar to those studied here may be used for reforestation purposes in the foothills with little risk of inbreeding depression. Although statistically significant tree-to-tree variation in outcrossing rates was encountered in only one of three stands studied, it may be wise to proceed with caution when using estimates of additive genetic variance and gain obtained through wind-pollinated lodgepole pine progeny tests. The magnitude of the tentative temporal trend observed in this study is not great enough to suggest that preference be given to older seed for reforestation or testing purposes.

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